AD_____

Award Number: W81XWH-05-1-0128

TITLE: Preclinical Testing the Therapeutic Potential of a Potent and Novel Small-molecule Inhibitor of Bcl-2 as a Novel Therapy for Hormone-refractory Prostate Cancer

PRINCIPAL INVESTIGATOR: Shaomeng Wang, Ph.D.

CONTRACTING ORGANIZATION: Regents of the University of Michigan
Ann Arbor MI 48109-1274

REPORT DATE: December 2006

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| **1. REPORT DATE** *(DD-MM-YYYY)* | **2. REPORT TYPE** | **3. DATES COVERED** *(From - To)* |
|---|---|---|
| 01-12-2006 | Annual | 30 Nov 05 – 29 Nov 06 |

**4. TITLE AND SUBTITLE**
Preclinical Testing the Therapeutic Potential of a Potent and Novel Small-molecule Inhibitor of Bcl-2 as a Novel Therapy for Hormone-refractory Prostate Cancer

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-05-1-0128

**5c. PROGRAM ELEMENT NUMBER**

**6. AUTHOR(S)**
Shaomeng Wang, Ph.D.

E-Mail: shaomeng@umich.edu

**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**

Regents of the University of Michigan
Ann Arbor MI 48109-1274

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
Targeting the anti-apoptotic Bcl-2 members using non-peptide, small-molecule inhibitors is a new and exciting therapeutic strategy. Our work has led to the discovery of potent, non-peptide small-molecule inhibitor apogossypolone that not only binds to Bcl-2 and Bcl-xL proteins but also Mcl-1. Consistent with its strong binding affinity to Bcl-2 members, apogossypolone potently and effectively inhibits cancer cell growth in androgen-independent human prostate cancer PC-3 and DU-145 cell lines. Apogossypolone is well-tolerated in animals and has an excellent oral bioavailability. Extensive in vivo studies are being carried out to determine its antitumor activity in animal models of human prostate cancer. Apogossypolone may have a great therapeutic potential to be developed as an effective and non-toxic new therapy for the treatment of advanced, androgen-independent human prostate cancer.

**15. SUBJECT TERMS**
None provided.

| **16. SECURITY CLASSIFICATION OF:** | | | **17. LIMITATION OF ABSTRACT** | **18. NUMBER OF PAGES** | **19a. NAME OF RESPONSIBLE PERSON** |
|---|---|---|---|---|---|
| **a. REPORT** | **b. ABSTRACT** | **c. THIS PAGE** | | | USAMRMC |
| U | U | U | UU | 11 | **19b. TELEPHONE NUMBER** *(include area code)* |

**Table of Contents**

**Introduction**: Current chemotherapy has limited effectiveness in hormone-refractory prostate cancer and new therapeutic strategies are urgently needed to improving the survival and quality of life in patients with hormone-refractory prostate cancer.

Current chemotherapeutic agents most commonly work by directly or indirectly inducing apoptosis, or programmed cell death in tumor cells. The impaired ability of prostate cancer cells to undergo apoptosis plays a key role in the resistance of prostate cancer cells to chemotherapy or radiation and for the failure of current treatment protocols for hormone-refractory prostate cancer. Hence, current and future efforts for designing new therapies to treat hormone-refractory prostate cancer must include strategies that specifically target resistance of prostate cancer cells to apoptosis.

Bcl-2 is a potent cellular inhibitor of apoptosis. Bcl-2 is overexpressed in 30-60% of prostate cancer at diagnosis but in nearly 100% of hormone-refractory prostate cancer. Prostate cancers that express high level of Bcl-2 are often resistant to chemotherapeutic agents or radiation therapy. Therefore, overexpression of Bcl-2 may play an important role to the high failure rate for current treatment of hormone-refractory prostate cancer. Hence, inhibition of the anti-apoptotic function of Bcl-2 represents a promising strategy for overcoming the resistance of prostate cancer to chemotherapy or radiation therapy and for developing an entirely new class of anticancer drugs for treatment of prostate cancer, especially hormone-refractory prostate cancer.

In this idea grant, we have proposed to test a potent and novel small-molecule inhibitor that we have discovered and synthesized in our laboratory for its mechanism of action and therapeutic potential for the treatment of human prostate cancer.

**Body of the report:**

**Task 1**. Development of an efficient synthetic procedure for the synthesis of our target compound, apogossypolone. Using this method, we have synthesized gram quantity of apogossypolone for our proposed *in vitro* and *in vivo* studies. We have further determined that in contrast to its parent compound gossypol, which has stable (+)- and (-)-gossypol (two enantiomers), apogossypolone has a single stable isomer. **Task #1 is now completed.**

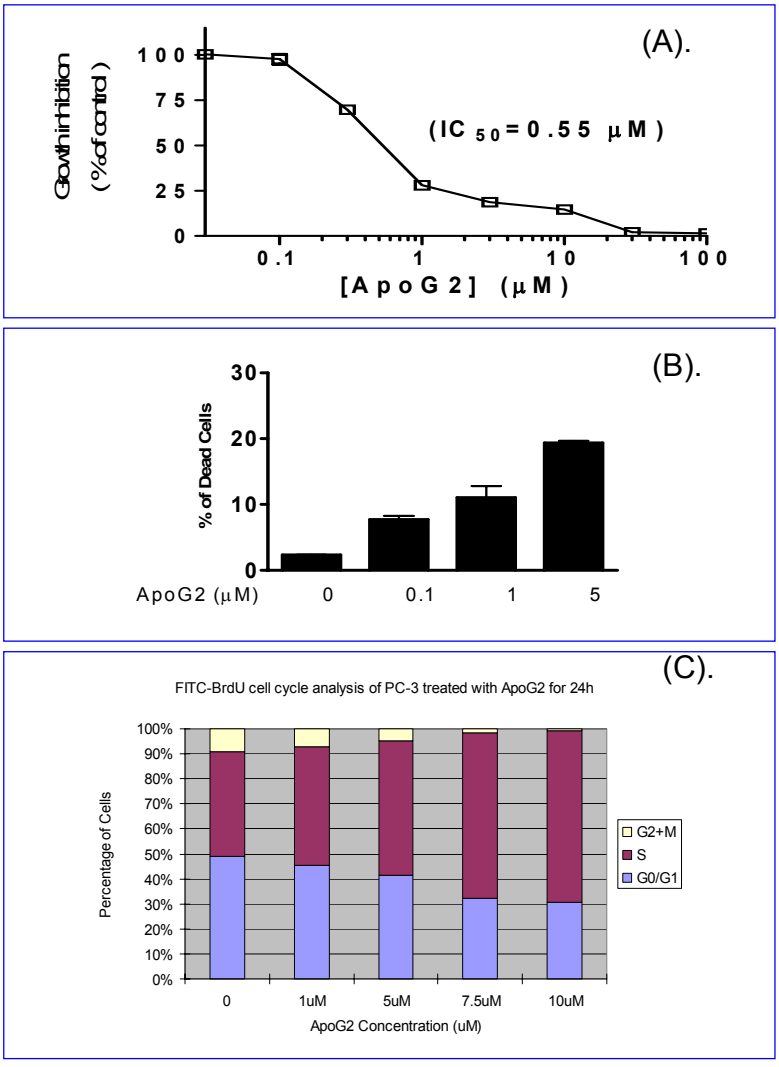
**Task 2**. In addition to our Bcl-2 and Bcl-xL binding assays, we have developed a fluorescence-polarization binding assay for Mcl-1, a member of anti-apoptotic Bcl-2 proteins. We have determined the binding affinities of apogossypolone to Bcl-2, Bcl-xL and Mcl-1 proteins using these sensitive and quantitative assays and shown that apogossypolone binds to Bcl-2, Bcl-xL and Mcl-1 proteins with Ki values of 170, 660 and 51 nM, respectively. Therefore, apogossypolone is a potent small-molecule inhibitor against multiple Bcl-2 proteins. Recently studies on inhibitors that specifically target Bcl-2 and Bcl-xL show that such inhibitors (e.g. ABT-737 developed from Abbott's Laboratories) are only active in a small subset of human cancer cell lines with low levels of Mcl-1 protein. Down-regulation of Mcl-1 can dramatically sensitize cancer cells to ABT-737. Hence, apogossypolone, which targets not only Bcl-2 and Bcl-xL proteins, but also Mcl-1, may have a major advantage over inhibitors that only target Bcl-2 and Bcl-xL and spare Mcl-1 protein. **Task #2 is now completed.**

**Task 3**. In our last report, we showed that apogossypolone (ApoG2) is active in inhibition of cell growth in androgen-independent PC-3 and DU-145 prostate cancer cell lines. We have now performed further analysis on apogossypolone in the PC-3 cancer cell line and the results are summarized in **Figure 1.**

Our data showed that ApoG2 can induce significant cell death in PC-3 cancer cells (Figure 1B) at as low as 100 nM concentration, consistent with its strong binding affinity to Bcl-2/Bcl-xL/Mcl-1 proteins. Very interestingly, our cell cycle analysis showed

that Apog2 has a very strong cell cycle effect (Figure 1C) and causes blockage from G1 phase transition to G2/M phase. Therefore, the very potent activity by ApoG2 in cell growth inhibition in PC-3 cells is a combination of cell death induction and cell cycle arrest. We are currently investigating what is the primary mechanism that mediates its activity in apoptosis and cell cycle arrest.

**Figure 1**. Determination of the activity of Apogossypolne (ApoG2) in the androgen-independent PC-3 human prostate cancer cell line (A): Inhibition of cell growth; Cells were treated by ApoG2 for 4 days and cell growth was determined using a WST-based assay. (B). Induction of cell death in PC-3 cells by ApoG2. Cells were treated by ApoG2 for 4 days and cell viability was determined using trypan blue exclusion assay. (C). Cell cycle analysis. Cells were treated by ApoG2 for 24 hours and cell cycle was performed by flow cytomertric analysis using FITC-BrdU labeling.



**Task 5.** We will investigate their synergistic activity in combination with chemotherapeutic agents *in vitro.*

We are in the process of performing extensive in vitro studies for the combination of ApoG2 with multiple chemotherapeutic agents. These include cell growth and cell death induction assays in PC-3 and DU-145 cancer cell lines. This task should be complete within the next 4-8 weeks.

**Task 6.** We will investigate their *in vivo* antitumor activity and specificity in human prostate cancer xenograft models (PC-3 and DU-145) and to examine for any sign of toxicity.

For *in vivo* anti-tumor activity studies, it is essential to determine the maximum tolerated doses (MTD) before efficacy experiments can be carried out. To this end, we have tested apogossypolone extensively in mice for its maximum tolerated dose (MTD). It was found that apogossypolone was well tolerated. Mice dosed at 80 mg/kg intravenously daily for 2 weeks showed no weight loss or other signs of toxicity. Furthermore, mice dosed at 240 mg/kg *via* oral gavage (oral dosing) daily for 2 weeks also did not show weight loss or other signs of toxicity. In direct comparison to gossypol, apogossypolone is at least 5-10 times more tolerated in mice. Our data thus suggested that apogossypolone is well tolerated in mice and has a much reduced toxicity as compared to its parent compound gossypol, consistent with our initial prediction that removal the two aldehyde groups in gossypol will greatly reduce the toxicity of this compound to animals.

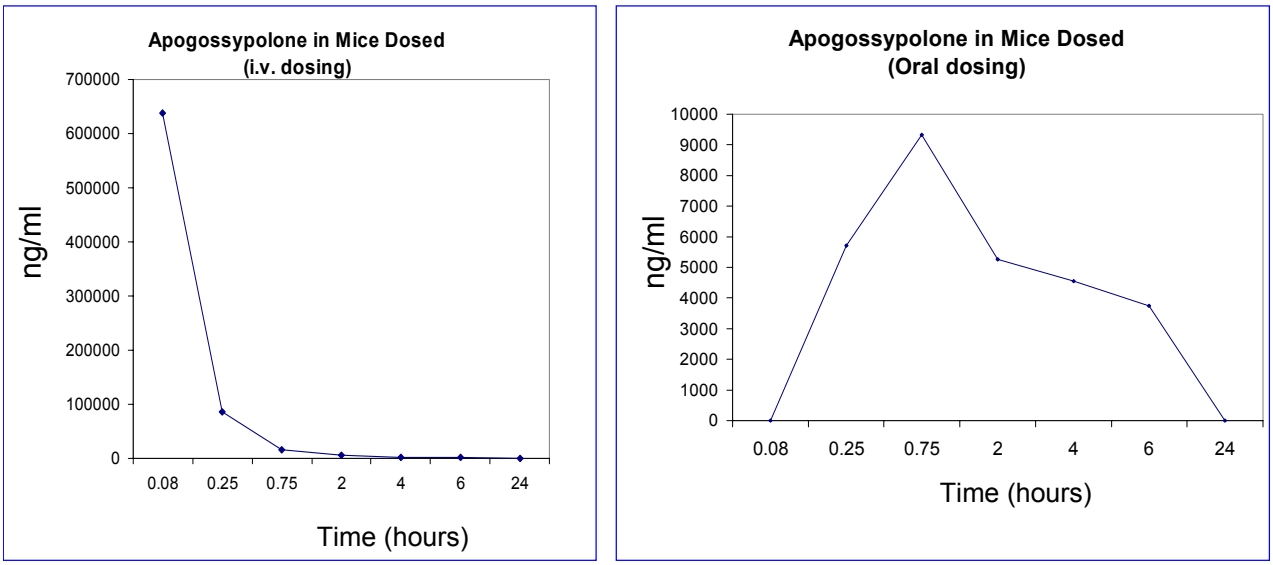
We next determined the pharmacokinetics (PK) of apogossypolone to assess its bioavailability. Since it is highly desirable to develop an orally available anticancer drug, we have performed our PK studies using both i.v. and oral routes of administration of the drug. The data are summarized in **Figure 2**.

As can be seen, apogossypolone has a good PK profile in oral dosing. At 30 mg/kg, apogossypolone has a cMax of 9000 ng/ml (18 $\mu$M since the molecular weight of apogossypolone is 492.5). Although its $T_{1/2}$ is only 1 hour in oral dosing, very high

concentration of the drug was observed 6 hours after the dosing with a concentration of 3500 ng/ml (7 μM).

Taken together, our toxicity and pharmacokinetic studies demonstrate that apogossypolone is well-tolerated in mice and has a good pharmacokinetic profile when given orally. Our studies laid the solid foundation for our subsequent in vivo efficacy studies to determine its antitumor activity in animal models of androgen independent, human prostate cancer.

**Figure 2**. Determination of the pharmacokinetics of apogossypolone in mice. A group of mice were dosed with a single dose of apogossypolone at 30 mg/kg and plasma were collected at 0 min, 5, 15, 45 min, and 2, 4, 6 and 24 hours. Each group consisted of 2 mice. Samples were analyzed using a highly sensitive LC-MS-MS method developed for apogossypolone.



**Key Research Accomplishments in year 2**:

(1). We have demonstrated that apogossypolone is effective and potent in induction of cell death in the PC-3 androgen independent prostate cancer cell line. Interestingly, we also showed that apogossypolone induces cell cycle arrest in PC-3 cancer cells. Therefore, the potent activity of apogossypolone in inhibition of cell growth in prostate cancer cells is a combination of cell death induction and cell cycle arrest.

(2). We have determined that apogossypolone is well tolerated in mice in both i.v. dosing and oral dosing. At the highest doses we can give to animals with the current formulation, no toxicity was observed for apogossypolone.

(3). We have determined the pharmacokinetics of apogossypolone in mice. Our pharmacokinetic studies showed that apogossypolone has an excellent oral bioavailability and pharmacokinetic profile.

Our work in year 2 laid the solid foundation for the proposed extensive *in vivo* studies to be performed in year 3.

**Reportable Outcomes:**

(1). A manuscript described the design, synthesis and initial evaluation of apogossypolone as potent small-molecule inhibitors of Bcl-2/Bcl-xL/Mcl-1 has been completed and will be submitted soon.

(2). Second manuscript on detailed *in vitro* and *in vivo* evaluations of apooysspolone in human prostate cancer models will be prepared and submitted.

(3). A patent application has been filed on the discovery of apooysspolone and methods of use for the treatment of human prostate and other types of cancer.

**Conclusions**: Targeting the anti-apoptotic Bcl-2 members using non-peptide, small-molecule inhibitors is a new and exciting therapeutic strategy. Our work has led to the discovery of potent, non-peptide small-molecule inhibitor apogossypolone that not only binds to Bcl-2 and Bcl-xL proteins but also Mcl-1. Consistent with its strong binding affinity to Bcl-2 members, apogossypolone potently and effectively inhibits cancer cell growth in androgen-independent human prostate cancer PC-3 and DU-145 cell lines. We demonstrated that apogossypolone effectively induces cell death and also cell cycle arrest. Mostly importantly, we have determined that apogossypolone is well-tolerated in animals and has an excellent oral bioavailability and pharmacokinetic profiles. Extensive in vivo studies are ongoing to further determine the antitumor activity of apogossypolone in animal models of human prostate cancer. Apogossypolone represents a highly promising, orally available, potent small-molecule inhibitor of Bcl-2/Bcl-xL/Mcl-1 to be developed for the treatment of advanced, androgen-independent human prostate cancer.